UNIVERSITI TEKNOLOGI MARA

CHITINOPHILIC AND KERATINOPHILIC FUNGI AS A SOURCE OF SECONDARY METABOLITES

SITI NUR SARAH BINTI ZUBIR

Thesis submitted in fulfilment
of the requirements for the degree of
Master of Science

Faculty of Pharmacy

August 2017

ABSTRACT

Fungi, recognised as frequent producers of secondary metabolites, occupy virtually all possible ecological niches. The present study focuses on studying chitinophilic and keratinophilic fungi as a potential source of secondary metabolites. The main objective of this study was to isolate secondary metabolites from the above-mentioned fungi using a modified version of the protocol named MECSUS (Microtiter plate, Elicitors, Combination, Solid phase extraction, UHPLC, Statistical analysis), which was recently developed in the Microbial Metabolite Laboratory of Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns). Putative chitinophilic fungi were isolated by collecting insects that were sick or dead and showing signs of fungal infection, while presumed keratinophilic fungi were obtained using the 'Tokava' hair-baiting method on soil samples collected from the Biological Research of AuRIns at Puncak Alam (Selangor), a fish sanctuary at Sungai Chilling (Selangor), the Endau-Rompin National Park (Johor) and Tanah Aina at Bentong (Pahang). Fifteen fungi were isolated, namely *Penicillium sp.* (TOWB-F2), *Trichoderma virens* (SA-F1), *Gliomastix polychroma* (SC14a-1), *Fusarium solani* (SC14a-2), *Penicillium sp.* (SC14b-1), *Pseudallescheria boydii* (ERS), *Fusarium decemcellulare* (ERI), *Boeremia exigua* (ER2a-1), *Nigrospora oryzae* (ER2a-2), *Wardomyces moseri* (ER2b-1), and *Purpureocillium lilacinum* (BENTONG) and unidentified species (TOWB-F1, TOWB-F3, 5FS-F1, TOH). A growth study was conducted over a month on the first five isolated fungi (i.e. TOWB-F1, TOWB-F2, TOWB-F3, SA-F1 and 5FS-F1) to determine the suitable duration of fermentation. The isolated fungi were grown simultaneously on 96-well microtiter plates in 8 media made of a common standard composition and supplemented with various elicitors. Liquid-liquid extraction was used to extract the secondary metabolites from the cultures. The crude extracts were analysed by HPLC. Selected extracts were fractioned until pure compound were obtained. Spectroscopic analysis was performed using nuclear magnetic resonance (NMR), ultraviolet (UV), and mass spectrometry (MS) to elucidate the structure of pure compounds. Four compounds were isolated during this study that is penicillic acid, pseurotin A, patulin and javanicin.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful

First and foremost, Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. I would like express my sincere gratitude and appreciate to my supervisor Prof Dr Jean-Frédéric Faizal Weber Abdullah for the continuous support of master study and research, for patience, motivation, enthusiasm, and immense knowledge. I could not have imagined having a better advisor and mentor for my master study. I would like to thank the rest of my thesis committee, Dr Nurhuda Manshoor and Dr Sadia Sultan. This research could not have been achieved without the assistance and support that I received.

I would like to thank to the members of my department, Faculty of Pharmacy: all technicians and office staffs for their cooperation.

I would like to take this opportunity to thank Associate. Prof. Dr. Anthony Cole from University Canterbury for his assistance and advice. Thank you to all AuRIns's members, laboratory staffs and postgraduate students for their continuous support during my study. Much of my experimental work would have not been completed without the assistance of my bench work friends: Fatimah Bebe, Rohani, Ummu Iffah, Siti Hajar, Fatmawati, Sharifah Nurfazilah, Nur Amina, Fatimah Wahid, Yusnita Alwia, Farhana, Mahanim, Ainnur Marlyana, Rohaity and everyone who have contributed to this research.

Deepest thanks to beloved family who has given me infinite support and constant encouragement throughout my research. With thank to my parents who allowed me to dream. To my father Zubir Mohd Yusof, I owe him a deep sense of gratitude for his endless prayer. To my mother, thank you for never giving up on me. Thank you very much.

TABLE OF CONTENTS

CHAPTER TWO
LITERATURE REVIEW

2.1 SECONDARY METABOLITES

Secondary metabolites are small natural products that do not play an obvious role in the basic cellular function but help the organism to adapt to its environment niche (O'Brien and Wright, 2011). Natural products are important in both drug discovery and chemical biology. They are privileged structures with structural motifs capable of interacting with variety of unrelated molecular targets (Nicolaou *et al.*, 2000). This ability is important in ecological chemical interaction and, thus, can play a major role in combating many human and animal diseases (Frisvad *et al.*, 2008; Müller, 2001; Vicente *et al.*, 2003). According to Newman and Cragg (2012) from 1981 to 2010, approximately half of approved drugs are either natural products, or directly or indirectly derived from them. Natural products have been utilized as sources of novel structures but not necessarily as final drugs. In the areas of cancer and infectious diseases, over 60% and 75% of these drugs were shown to be of natural origin (Newman *et al.*, 2003). Generally, there are three groups of organisms that are particularly good producers of secondary metabolites, namely plants, fungi (including lichen fungi) and actinobacteria. Other organisms, for example yeasts, protozoa and animals are less efficient producers (Frisvad *et al.*, 2008). They are considered as less efficient as they are very difficult to obtain in pure cultures compared to plants and fungi. For example myxomycetes, myxobacteria and cyanobacteria are discovered as a good producers. However, the development of methods for the cultivation and maintenance of these organisms remaining a crucial issue (Gaspari *et al.*, 2005; Nunnery *et al.*, 2010; Steglich, 1989).

The medicinal use of natural products deriving from sources such as plants, animals, or microorganisms precedes recorded human history by probably tens of thousands of years. Indigenous people have a very long history of using natural ingredients for medicinal purposes. Traditional Chinese medicine is also famous for its extensive use of herbs. The monograph Shen Nong Ben Cao Jing (Shen Nong Materia Medica) was compiled during the Eastern Han dynasty and documented 365